began to diverge in their fixation patterns. While low-risk infants generally maintained or increased their looking toward the eyes, decreasing fixation of the eyes within a face was observed in those who went on to later autism.

From these results, Jones and Klin [9] conclude that basic mechanisms supporting social orienting toward the eyes are not diminished in the first months after birth, but begin to decline thereafter. Further, while in typical development newborns orienting to social stimuli increases the exposure of developing cortical circuits to these biologically important stimuli, thus facilitating the specialization of the cortical social brain [7], the authors speculate that this canalization is disrupted in those who go on to autism.

Given that these recent results indicate that basic social orienting is unaffected in autism, how are we to explain the fact that under many circumstances most individuals with autism spend less time engaged in interaction with others, show atypical fixation patterns towards the eyes, and are generally poor at extracting complex information from faces (such as emotions and intention)?

One line of thinking is that while the subcortical route may be intact in autism, its interactions with the cortical social brain network are aberrant. For example, Senju [11] has advanced a specific model of how engaging the sub-cortical route (through presentation of a face with direct gaze) modulates the activation of cortical regions that are part of the social brain network. Research is currently underway in children and adults with autism to test if there are differences in the interaction between the cortical and subcortical systems.

A second line of thinking is that it is the cortical structures of the human social brain network that are impaired. This approach, in turn, falls in to two broad camps, with one group of investigators pointing to evidence for a lack of specialization of specific cortical regions such as the temporal-parietal junction [12], and others who hypothesise that a widespread synaptic problem across large swathes of cortex may differentially affect our processing of other humans, as these are the most complex, dynamic and unpredictable aspects of the external environment [13].

Future studies of the early neurodevelopment of autism will be critical in resolving these issues, the significance of which goes well beyond academic debate. Unravelling cause from effect around the age of onset of autism may allow us to design targeted interventions before atypical processing embeds itself as a life-long condition.

References

1. Senju, A., and Johnson, M.H. (2009). Atypical eye contact in autism: Models, mechanisms, and development. Neurosci. Biobehav. Rev. *33*, 1204–1214.
2. Jones, W., Carr, K., and Klin, A. (2008). Absence of preferential looking to the eyes of approaching adults predicts level of social disability in 2-year olds with autism spectrum disorder. Arch. Gen. Pysch. *65*, 946–954.
3. Dawson, G., Webb, S.J., and McPartland, J. (2005). Understanding the nature of face processing impairment in autism: insights from behavioral and electrophysiological studies. Dev. Neuropsychol. *27*, 403–424.
4. Schultz, R.T. (2005). Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. Int. J. Dev. Neurosci. *23*, 125–141.
5. Johnson, M.H., Dziurawiec, S., Ellis, H.D., and Morton, J. (1991). Newborns preferential tracking of face-like stimuli and its subsequent decline. Cognition *40*, 1–19.
6. Farroni, T., Csibra, G., Simion, F., and Johnson, M.H. (2002). Eye contact detection in humans from birth. Proc. Natl. Acad. Sci. USA *99*, 9602–9605.
7. Johnson, M.H. (2005). Sub-cortical face processing. Nat. Rev. Neurosci. *6*, 766–774.
8. Shah, P., Gaule, A., Bird, G., and Cook, R. (2013). Robust orienting to protofacial stimuli in autism. Curr. Biol. *23*, R1087–R1088.
9. Jones, W., and Klin, A. (2013). Attention to eyes in present but in decline in 2-6 month olds later diagnosed with autism. Nature http://dx.doi.org/10.1038/nature12715.
10. Tomalski, P., Csibra, G., and Johnson, M.H. (2009). Rapid orienting toward face-like stimuli with gaze-relevant contrast information. Perception *38*, 569–578.
11. Senju, A., and Johnson, M.H. (2009). The eye contact effect: Mechanisms and development. Trends Cogn. Sci. *13*, 127–134.
12. Pelphrey, K.A., Shultz, S., Hudac, C.M., and Vander Wyk, B.C. (2011). Constraining heterogeneity: The social brain and its development in autism spectrum disorder. J. Child Psych. Psych. *52*, 631–644.
13. Dinstein, I., Heeger, D.J., Lorenzi, L., Minshew, N.J., Malach, R., and Behrmann, M. (2012). Unreliable evoked responses in autism. Neuron *75*, 981–991.

Centre for Brain and Cognitive Development, Henry Wellcome Building, Birkbeck, University of London, London WC1E 7HX, UK.
E-mail: Mark.johnson@bbk.ac.uk

http://dx.doi.org/10.1016/j.cub.2013.11.021

Actin Dynamics: Cell Migration Takes a New Turn with Arpin

Accurate cell migration requires intricate control over the actin cytoskeleton. Recent work has identified an Arp2/3-interacting protein called Arpin, which restricts the rate of actin polymerization and is the latest component in the steadily expanding protein repertoire that controls cell migration.

Douwe Veltman

The eukaryotic cell depends on its actin cytoskeleton for normal growth and development. The cytoskeleton provides the force that is necessary for essential cellular processes, such as cytokinesis, phagocytosis or lamellipodia formation. These structures are major sites of actin filament nucleation and typically display highly nonlinear kinetics, meaning that they are sharply defined in both space and time. How these nonlinear processes are regulated remains poorly understood.

A clue to the origin of the nonlinearity lies in the nature of one of the most important nucleators of new actin filaments — the Arp2/3 complex. The Arp2/3 complex nucleates actin filaments by binding to the side of an existing filament and initiating branch formation. The resulting two new filaments can then each be split again, creating a natural feed-forward mechanism, limited only by the supply of components, such as Arp2/3 complex, actin monomers, and activators, most of which diffuse from the bulk cytoplasm.

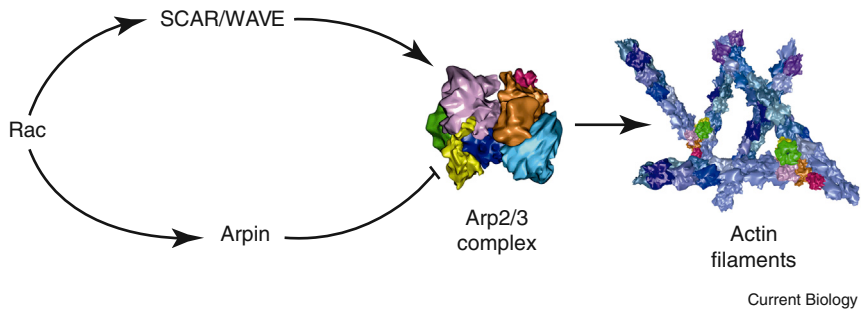

Figure 1. Schematic pathway of actin polymerization.

Rac simultaneously activates actin polymerization via the SCAR/WAVE complex and inhibits actin polymerization via Arpin, potentially resulting in an incoherent feed-forward loop. Protein models of the Arp2/3 complex and F-actin were created in Blender (Blender Foundation, Amsterdam) based on the crystal structures solved in [12] and [13], respectively.

Without any negative regulation, such positive feedback can quickly spiral out of control, but how nucleation activity is precisely regulated is not well understood. In a recent *Nature* paper, Dang *et al.* [1] report the identification of an as yet uncharacterized Arp2/3-interacting protein that they have called Arpin. These authors present evidence that Arpin is a negative regulator of Arp2/3 activity and that cells utilize Arpin to fine-tune actin nucleation activity at the leading edge of the lamellipodium to steer the cell [1].

Dang *et al.* [1] performed a bioinformatics search for proteins containing a highly conserved carboxy-terminal Arp2/3-binding motif. The classical Arp2/3-binding domain consists of an amphipathic α-helix, sometimes referred to as the coil region, that binds to the barbed end groove of Arp2, followed by a short acidic motif that has a highly conserved tryptophan residue at position –1 or –2 relative to the carboxyl terminus [2]. The acidic motif is thought to bind to Arp3, but its exact binding position remains debated [3]. In WASP family proteins, which are the main activators of Arp2/3, this domain is preceded by an actin monomer binding motif, making it ideally suited to deliver the first actin monomer to the Arp2/3 complex, thereby initiating a new branch.

The newly identified protein, Arpin, contains a prototypical acidic motif, but no amphipathic α-helix or actin monomer binding domain. Despite the lack of an amphipathic α-helix, biochemical and immunological experiments proved that Arpin indeed binds to the Arp2/3 complex. The lack of an actin monomer binding domain does not necessarily exclude a function as an activator of the Arp2/3 complex, as some atypical activators function without a recognizable actin monomer binding domain [4]. Using a combination of standard pyrene-labeled actin polymerization assays and TIRF microscopy, however, Dang *et al.* [1] were able to confirm that Arpin does not activate the Arp2/3 complex. Instead, binding of Arpin to the Arp2/3 complex inhibits actin filament nucleation, making it a competitive Arp2/3 inhibitor.

With these validations in place, Arpin now joins a short list of mammalian proteins with true carboxy-terminal acidic Arp2/3-binding motifs. The identification of a putative biological role remains non-trivial because many regulators of actin filament turnover have cryptic functions. Just like Arpin, coronin7 proteins have a highly conserved carboxy-terminal acidic motif, but their exact function has not yet been established [5]. In mammalian cells coronin7 is recruited to the Golgi where it helps maintain its proper morphology, whereas the coronin7 homologue of the social amoeba *Dictyostelium* is recruited to crown-like structures associated with fluid-phase endocytosis and disruption of coronin7 leads to an increase in phagocytosis [6]. Another protein with a conserved carboxy-terminal acidic motif is the poorly studied WAFL protein, which is a marker for the inflammatory bowel disease ulcerative colitis. Initial pilot experiments have led to the suggestion that WAFL participates in innate immune functions, but it may also have roles in endocytosis and vesicle trafficking as it interacts with both the AP2 complex and with the WASH complex, the major Arp2/3 activator at the surface of endosomes [7,8].

The quest to determine the biological function of Arpin was aided by the observation that the protein is recruited to the leading edge of lamellipodia during migration of mouse embryonic fibroblasts. Interestingly, Arpin levels are inversely correlated with lamellipodium speed and persistence. Deletion of the Arpin homologue in *Dictyostelium* amoebae results in cells exploring a wider territory with both higher cell speed and higher directional persistence. The fact that the observed phenotypic effects are conserved across evolution is an excellent indication that steering of the cell by inhibition of pseudopodia/lamellipodia formation is a true physiological function of Arpin.

Clues to the mechanism by which Arpin controls lamellipodia formation were gained from its mode of recruitment to the leading edge. Recruitment of Arpin to lamellipodia is dependent on active Rac; in Rac1 knockout fibroblasts Arpin no longer localises to the leading edge and it no longer co-immunoprecipitates with the Arp2/3 complex. Initially these findings appear counterintuitive, as active Rac is also a potent activator of lamellipodia formation by binding to and activating the SCAR/WAVE complex, which in turn is the principal activator of the Arp2/3 complex in lamellipodia [9]. However, the findings agree with a number of models that have been developed to explain cell motility and directional migration. These models use what is called an ‘incoherent feed-forward loop’ (Figure 1). It is generally accepted that the strong accumulation of actin nucleation factors at the leading edge depends on positive feedback. To prevent feed-forward loops from locking into a permanently activated state, a negative feedback loop can be introduced that has relatively slow turnover or that requires high concentrations of activator. Such parallel loops of fast activation and delayed inhibition create periodic cycles of activation and inactivation that do not reach equilibrium.

A popular model that uses an incoherent feed-forward loop to explain cell migration was proposed by Hans Meinhardt [10] and has recently

been used in computer-assisted simulations that resulted in realistically migrating cells [11]. Despite their innate beauty, validation of these models depends on the identification of the molecular components that represent the parameters of the model; in particular, the so-called local inhibitor that facilitates the negative feedback has remained elusive. Arpin is now a prime candidate for this local inhibitor, given that it is a competitive inhibitor of actin filament nucleation and has been shown experimentally to contribute to the collapse of lamellipodia.

The current research opens up various new avenues of research. One of immediate interest is whether loss of Arpin function is associated with increased cell motility in epithelial–mesenchymal transition during the progression of cancer. The inhibitory effect of Arpin on cell migration could potentially be used to control metastasis. Another open question is whether the steering of the cells is coupled to the gradient-sensing machinery. Bacterial cells chemotax by inducing turns in decreasing concentrations of chemoattractant and by persisting in their current direction when the concentration of chemoattractant increases. Arpin-mediated control of migratory persistence might be utilized to direct eukaryotic cells in a similar manner.

With the ever-growing number of genomic sequences, database searches are an increasingly successful way to identify new protein interactions that have thus far remained undetected using biochemical or immunological assays and it remains to be seen how many new proteins that are involved in cell migration can be identified. Speed is of the essence though, as by my count, the number of mammalian proteins with undescribed acidic carboxy-terminal motifs has now decreased to one.

References

1. Dang, I., Gorelik, R., Sousa-Blin, C., Derivery, E., Guerin, C., Linkner, J., Nemethova, M., Dumortier, J.G., Giger, F.A., Chipysheva, T.A., *et al*. (2013). Inhibitory signalling to the Arp2/3 complex steers cell migration. Nature *503*, 281–284.
2. Boczkowska, M., Rebowski, G., Petoukhov, M.V., Hayes, D.B., Svergun, D.I., and Dominguez, R. (2008). X-ray scattering study of activated Arp2/3 complex with bound actin-WCA. Structure *16*, 695–704.
3. Ti, S.C., Jurgenson, C.T., Nolen, B.J., and Pollard, T.D. (2011). Structural and biochemical characterization of two binding sites for nucleation-promoting factor WASp-VCA on Arp2/3 complex. Proc. Natl. Acad. Sci. USA *108*, E463–E471.
4. Wagner, A.R., Luan, Q., Liu, S.L., and Nolen, B.J. (2013). Dip1 defines a class of Arp2/3 complex activators that function without preformed actin filaments. Curr. Biol. *23*, 1990–1998.
5. Chan, K.T., Creed, S.J., and Bear, J.E. (2011). Unraveling the enigma: progress towards understanding the coronin family of actin regulators. Trends Cell Biol. *21*, 481–488.
6. Shina, M.C., Unal, C., Eichinger, L., Muller-Taubenberger, A., Schleicher, M., Steinert, M., and Noegel, A.A. (2010). A Coronin7 homolog with functions in actin-driven processes. J. Biol. Chem. *285*, 9249–9261.
7. Pan, Y.F., Viklund, I.M., Tsai, H.H., Pettersson, S., and Maruyama, I.N. (2010). The ulcerative colitis marker protein WAFL interacts with accessory proteins in endocytosis. Int. J. Biol. Sci. *6*, 163–171.
8. Viklund, I.M., Kuznetsov, N.V., Lofberg, R., Daperno, M., Sostegni, R., Astegiano, M., Rizzetto, M., von Stein, O., D'Amato, M., von Stein, P., *et al*. (2008). Identification of a new WASP and FKBP-like (WAFL) protein in inflammatory bowel disease: a potential marker gene for ulcerative colitis. Int. J. Colorect. Dis. *23*, 921–930.
9. Veltman, D.M., King, J.S., Machesky, L.M., and Insall, R.H. (2012). SCAR knockouts in Dictyostelium: WASP assumes SCAR's position and upstream regulators in pseudopods. J. Cell Biol. *198*, 501–508.
10. Meinhardt, H. (1999). Orientation of chemotactic cells and growth cones: models and mechanisms. J. Cell Sci. *112*, 2867–2874.
11. Neilson, M.P., Veltman, D.M., van Haastert, P.J., Webb, S.D., Mackenzie, J.A., and Insall, R.H. (2011). Chemotaxis: a feedback-based computational model robustly predicts multiple aspects of real cell behaviour. PLoS Biol. *9*, e1000618.
12. Robinson, R.C., Turbedsky, K., Kaiser, D.A., Marchand, J.B., Higgs, H.N., Choe, S., and Pollard, T.D. (2001). Crystal structure of Arp2/3 complex. Science *294*, 1679–1684.
13. Oda, T., Iwasa, M., Aihara, T., Maeda, Y., and Narita, A. (2009). The nature of the globular- to fibrous-actin transition. Nature *457*, 441–445.

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK.
E-mail: dveltman@mrc-lmb.cam.ac.uk

http://dx.doi.org/10.1016/j.cub.2013.11.022

Evolution: 'Snowed' In with the Enemy

Explaining the origins and maintenance of cooperation in nature is a key challenge in evolutionary biology. A recent study demonstrates two novel mechanisms through which the natural ecology of sinking ocean aggregates — colloquially called 'marine snow' — promotes cooperation.

Manoshi Sen Datta[1] and Jeff Gore[2,*]

It was 1930 when William Beebe, peering out from the porthole of his deep-sea bathysphere (Figure 1), first witnessed the ever-present shower of flocculent, rapidly sinking aggregates that we now know as 'marine snow' [1]. Composed of dead phytoplankton, zooplankton fecal pellets, and other nutrient-rich detritus, marine snow particles are hotspots of microbial activity in an otherwise barren ocean landscape (Figure 2) [2]. Indeed, as they descend through the water column, particles of marine snow are rapidly colonized by bacteria, which form dense biofilm communities on the particle surface [2,3]. In some cases, only a portion of bacterial colonizers can degrade the complex polysaccharides that comprise marine snow, but they often produce smaller, freely diffusing nutrients that can be consumed by the entire community [3,4]. However, producers of these shared nutrients can be taken advantage of by free-riding non-producers that consume the nutrients without paying the metabolic cost of contributing to the pool themselves [4]. How do 'cooperating' producers survive in the face of these 'cheating' non-producers in the marine snow environment? A new study in this issue of *Current Biology* by Drescher *et al.* [5] demonstrates that non-producers can indeed exploit producers in particle-associated bacterial communities, but also suggests novel mechanisms through which the natural ecology of marine snow may allow producers to subvert their free-riding neighbors.

To be sure, the question of how cooperating individuals can avoid exploitation by cheaters — often framed as the so-called 'public goods dilemma' — has interested evolutionary biologists for decades. Despite the costs of cooperation (for instance, the metabolic cost of producing carbohydrate-degrading